

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the above-identified application.

Claims:

Please cancel claims 2 and 6.

1. (Currently Amended) A method for synthesizing a nucleic acid molecule comprising at least one non-canonical nucleotide, comprising the steps of:
 - a) incubating a template nucleic acid in a reaction mixture under nucleic acid synthesis conditions containing (i) a double-mutant nucleic acid polymerase, wherein said polymerase has a reduced discrimination between canonical and non-canonical nucleoside triphosphates and wherein the polymerase is a T7 RNA polymerase comprising the mutation Y639F/H784A, and (ii) at least one non-canonical nucleoside triphosphate, wherein said non-canonical nucleoside triphosphate is incorporated into the synthesized nucleic acid in place of only one canonical nucleoside triphosphate, and
 - b) obtaining the synthesis of a nucleic acid molecule comprising at least one non-canonical nucleotide.
2. (Cancelled)
3. (Original) The method of claim 1 wherein the template nucleic acid is RNA.
4. (Original) The method of claim 1 wherein a nucleic acid molecule comprising at least one non-canonical nucleotide is synthesized by extension of a primer molecule, at least part of which is sufficiently complementary to a portion of the template to hybridize therewith.

5. (Original) The method of claim 1 wherein a nucleic acid molecule comprising at least one non-canonical nucleotide is synthesized de novo without using a primer molecule.
6. (Cancelled)
7. (Original) The method of claim 1 wherein the polymerase is a T7-type RNA polymerase.
8. (Original) The method of claim 1 wherein the polymerase is selected from the group consisting of T7 and T3 RNA polymerases.
9. (Original) The method of claim 1 wherein the double-mutant polymerase is an RNA polymerase and the non-canonical nucleoside triphosphate is a 2'-fluoro-nucleoside triphosphate.
10. (Cancelled)
11. (Currently Amended) The method of claim 1 wherein the synthesized nucleic acid molecule is selected from the group consisting of a ribozyme ~~or~~ and a nucleic acid molecule, wherein the molecule is used for gene therapy, in a vaccine, in an antiviral composition, in an antimicrobial composition, in an antisense composition for regulating gene expression, in a composition for hybridization to a complementary nucleic acid, or as a probe for detection of a complementary nucleic acid.
12. (Original) The method of claim 1 wherein the synthesized nucleic acid molecule is single-stranded.
13. (Withdrawn) A kit for performing the method of claim 1, comprising a double-mutant nucleic acid polymerase which has reduced discrimination

between canonical and non-canonical nucleoside triphosphates and data or information describing conditions under which the method of claim 1 may be performed.

14. (Withdrawn) The kit of claim 13, wherein the nucleic acid polymerase is a mutant T7-type RNA polymerase.

15. (Withdrawn) The kit of claim 13, wherein the nucleic acid polymerase is a T7 RNA polymerase comprising an altered amino acid at position 639 and 784.

16. (Withdrawn) The kit of claim 13, wherein the nucleic acid polymerase is SP6 RNA polymerase comprising an altered amino acid at position 631 and 779.

17. (Withdrawn) A method for determining the sequence of a nucleic acid molecule using a double-mutant RNA polymerase which has a reduced discrimination for non-canonical versus canonical nucleotides as substrates, comprising the steps of:

a) synthesizing a nucleic acid molecule de novo from an RNAP promoter sequence in a reaction mixture containing the mutant RNA polymerase in each of four separate reactions, each reaction comprising at least four nucleoside triphosphates, wherein at least one nucleoside triphosphate has a nucleic acid base which is complementary to each of adenine, cytidine, guanine and uracil or thymine and a sugar with either a hydroxy or a hydrogen or a fluorine at the 2'-position, and a portion of a ddNTP, such that each of the four separate reactions contains a ddNTP that is complementary to a different one of the four common nucleic acid bases in a nucleic acid molecule, and

b) evaluating the reaction products so that the sequence of the template molecule may be deduced.

18. (Withdrawn) The method of claim 17 wherein, each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of ATP, CTP, GTP, and UTP or rTTP, and further comprises one ddNTP.

19. (Withdrawn) The method of claim 17 wherein, each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of dATP, dCTP, dGTP, dUTP, dTTP, 7-deaza-dGTP, dITP, 5-methyl-dCTP, 5-hydroxymethyl-dCTP, and further comprises one ddNTP.

20. (Withdrawn) The method of claim 17 wherein, each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of 2'-F-ATP, 2'-F-CTP, 2'-F-GTP, 2'-F-UTP, 2'-F-TTP, 2'-deaza-2'-F-GTP, 2'-F-ITP, and 5-methyl-2'-F-CTP, 5-hydroxymethyl-2'-F-CTP and further comprises one ddNTP.

21. (Withdrawn) A method for determining the sequence of a nucleic acid molecule using a double-mutant RNA polymerase which has a reduced discrimination for non-canonical versus canonical nucleotides as substrates, comprising the steps of:

- a) synthesizing a nucleic acid molecule by extending a primer, wherein at least part of the primer is complementary to a template molecule so as to anneal therewith, in a reaction mixture containing the mutant RNA polymerase in each of four separate reactions, each reaction comprising at least four nucleoside triphosphates, wherein at least one nucleoside triphosphate has a nucleic acid base which is complementary to each of adenine, cytidine, guanine and uracil or thymine and a sugar with either an hydroxy or a hydrogen or a fluorine at the 2'-position, and further comprising a portion of a ddNTP, such that each of the four separate reactions contains a ddNTP that is complementary to a different one of the four common nucleic acid bases, and
- b) evaluating the reaction products so that the sequence of the template molecule may be deduced.

22. (Withdrawn) The method of claim 21 wherein, each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of ATP, CTP, GTP, and UTP or rTTP, and further comprises one ddNTP.
23. (Withdrawn) The method of claim 21 wherein, each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of dATP, dCTP, dGTP, dUTP, dTTP, 7-deaza-dGTP, dITP, 5-methyl-dCTP, 5-hydroxymethyl-dCTP and further comprises one ddNTP.
24. (Withdrawn) The method of claim 21 wherein, each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of 2'-F-ATP, 2'-F-CTP, 2'-F-GTP, 2'-F-UTP, 2'-F-TTP, 7-deaza-2'-F-GTP, 2'-F-ITP, and 5-methyl-2'-F-CTP, 5-hydroxymethyl-2'-F-CTP and further comprises one ddNTP.
25. (Original) The method of claim 1, wherein a dinucleotide or trinucleotide for initiation of de novo nucleic acid synthesis is added to the reaction mixture.
26. (Withdrawn) The method of claim 17, wherein at least one of the nucleoside triphosphates in the reaction mixture is modified to contain a radioactive or non-radioactive label.
27. (Withdrawn) The method of claim 17, wherein the ddNTP in the reaction mixture is modified to contain a radioactive or non-radioactive label.
28. (Original) The method of claim 25, wherein the dinucleotide or trinucleotide in the reaction mixture is modified to contain a radioactive or non-radioactive label.

29. (Withdrawn) A kit for performing a dideoxy sequencing reaction, comprising a double-mutant nucleic acid polymerase which has reduced discrimination between canonical and non-canonical nucleoside triphosphates and data or information describing conditions under which the method may be performed.

30. (Withdrawn) A method for determining the sequence of a nucleic acid molecule using a double-mutant RNA polymerase which has a reduced discrimination for non-canonical versus canonical nucleotides as substrates, comprising the steps of:

a) synthesizing a nucleic acid molecule de novo from an RNAP promoter sequence in a reaction mixture containing a mutant RNA polymerase in each of four separate reactions, each reaction comprising at least four nucleoside triphosphates, wherein at least one nucleoside triphosphate has a nucleic acid base which is complementary to each of adenine, cytidine, guanine and uracil or thymine and a sugar with a hydrogen or a fluorine at the 2'-position, and a portion of a rNTP, such that each of the four separate reactions contains a rNTP that is complementary to a different one of the four common nucleic acid bases, and

b) treating the nucleic acid products of the reactions so as to bring about hydrolysis of the phosphodiester backbone at all sites where a ribonucleotide has been incorporated, and

c) evaluating the reaction products so that the sequence of the template molecule may be deduced.

31. (Withdrawn) The method of claim 30 wherein the nucleic acid synthesis is part of or coupled to a method for nucleic acid amplification.

32. (Withdrawn) A kit for performing the method of claim 30 comprising a double-mutant nucleic acid polymerase which has reduced discrimination

between canonical and non-canonical nucleoside triphosphates and data or instructions describing conditions under which the method of claim 30 may be performed.

33. (Withdrawn) A kit for performing the method of claim 31 comprising a double-mutant nucleic acid polymerase which has reduced discrimination between canonical and non-canonical nucleoside triphosphates and data or instructions describing conditions under which the method of claim 31 may be performed.

34. (Withdrawn) A method for determining the sequence of a nucleic acid molecule using a double-mutant RNA polymerase which has a reduced discrimination for non-canonical versus canonical nucleotides as substrates, comprising the steps of:

a) synthesizing a nucleic acid molecule by extending a primer, at least part of which is sufficiently complementary to a template molecule so as to anneal therewith, in a reaction mixture containing a mutant RNA polymerase in each of four separate reactions, each reaction comprising at least four nucleoside triphosphates, wherein at least one nucleoside triphosphate has a nucleic acid base which is complementary to each of adenine, cytidine, guanine and uracil or thymine and a sugar with either a hydrogen or a fluorine at the 2'-position, and a portion of a rNTP, such that each of the four separate reactions contains a rNTP that is complementary to a different one of the four common nucleic acid bases in a nucleic acid molecule,

b) treating the nucleic acid products of the reactions so as to bring about hydrolysis of the phosphodiester backbone at all sites where a ribonucleotide has been incorporated, and

c) evaluating the reaction products using any of the methods common in the art for separating and detecting reaction products of sequencing reactions so that the sequence of the template molecule may be deduced.

35. (Withdrawn) The method of claim 34 wherein the nucleic acid synthesis is part of or coupled to a method for nucleic acid amplification.
36. (Withdrawn) A kit for performing the method of claim 34 comprising a double-mutant nucleic acid polymerase which has reduced discrimination between canonical and non-canonical nucleoside triphosphates and data or instructions describing conditions under which the method of claim 34 may be performed.
37. (Withdrawn) A kit for performing the method of claim 35 comprising a double-mutant nucleic acid polymerase which has reduced discrimination between canonical and non-canonical nucleoside triphosphates and data or instructions describing conditions under which the method of claim 35 may be performed.
38. (Original) The method of claim 1 wherein the nucleic acid synthesis is part of or coupled to a method for nucleic acid amplification.
39. (Withdrawn) A kit for performing the method of claim 38 comprising a double-mutant nucleic acid polymerase which has reduced discrimination between canonical and non-canonical nucleoside triphosphates and data or instructions describing conditions under which the method of claim 38 may be performed.
40. (Withdrawn) A kit for performing a partial ribo-substitution reaction comprising a double-mutant nucleic acid polymerase which has reduced discrimination between canonical and non-canonical nucleoside triphosphates and data or information describing conditions under which the method may be performed.

41. (New) A method for synthesizing a nucleic acid molecule comprising at least one non-canonical nucleotide, comprising the steps of:

a) incubating a template nucleic acid in a reaction mixture under nucleic acid synthesis conditions containing (i) a double-mutant nucleic acid polymerase, wherein said polymerase has a reduced discrimination between canonical and non-canonical nucleoside triphosphates and wherein the polymerase is selected from the group consisting of a T7 RNA polymerase, an SP6 polymerase, and a T3RNA polymerase (ii) at least one non-canonical nucleoside triphosphate, wherein said non-canonical nucleoside triphosphate is incorporated into the synthesized nucleic acid in place of only one canonical nucleoside triphosphate, and

b) obtaining the synthesis of a nucleic acid molecule comprising at least one non-canonical nucleotide.